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Determination of a permeability coefficient (Kp) for H-28308 using human and rat skin mounted in an in vitro static diffusion cell

This study was conducted under Work Request 17474, Service Code 1588.

MATERIAL AND METHODS

Samples of human and rat skin were dermatomed to approximately 450 µm and mounted onto an in vitro static diffusion cell (Figure 1). The donor and receptor chambers were filled with saline and the water-jacketed cells maintained at 32ºC using a re-circulating water bath. Following a brief equilibration, membrane integrity was confirmed using electrical impedance (n=3 replicates per species). Saline was then removed from the donor chamber and the test material H-28308, an aqueous solution of HFPO dimer acid ammonium salt (86%), which had been further diluted with water to a concentration of 124 mg/mL, was applied to the epidermal surface via the donor chamber as an infinite dose (pilot experiments had suggested application of the neat test substance would likely degrade the barrier properties of the skin, so a more dilute sample was used). The donor chamber was then occluded with Parafilm® and serial receptor fluid samples (100 µL) were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours and analyzed for HFPO anion by LC/MS/MRM (329>285 m/z). The cumulative amount of HFPO anion detected in the receptor fluid at each sampling time point was normalized to the exposure area (0.64 cm^2) and the results plotted as the cumulative amount penetrated (µg/cm^2) versus time (in hours) to produce a penetration profile. A permeability coefficient (Kp in cm/h) was calculated by dividing the penetration rate or slope of the line at steady-state (µg/cm^2/h) by the concentration of the applied chemical (µg/cm^3).

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RESULTS

(Tables 1-3, Figure 2)

Following application of an infinite dose of an aqueous dilution of H-28308 to dermatomed human and rat skin, the skin barrier properties had degraded by approximately 50% (independent of species) over the 24 hour contact time. Lag time was 1.73 ± 1.01 hours and 0.82 ± 0.77 hours for human and rat skin, respectively. Steady-state penetration was 6.2 ± 5.3 µg/cm²/h and 70 ± 5.3 µg/cm²/h for human and rat skin, respectively.

Based on the aqueous concentration of the applied test material (124 mg/mL) and steady-state penetration results, the Kp for human and rat skin was calculated to be $5.0 \times 10^{-5} \pm 4.3 \times 10^{-5}$ cm/h and $5.7 \times 10^{-4} \pm 4.3 \times 10^{-5}$ cm/h, respectively.

Table 1
Electrical Impedance (EI)

	Pre EI (k-ohms)		Post EI (k-ohms)		Ratio: Post/Pre	
	Mean	SD	Mean	SD	Mean	SD
Human	29.2	10.5	13.4	2.3	0.48	0.09
Rat	8.7	1.4	4.6	0.2	0.54	0.09

Table 2
Summary of kinetic parameters

		Mean	SD
Human	Lag time (hours)	1.73	1.01
	Penetration rate at steady-state (µg/cm²/h)	6.18	5.27
	Kp (cm/h)	5.02E-05	4.3E-05
Rat	Lag time (hours)	0.82	0.77
	Penetration rate at steady-state (µg/cm²/h)	70.3	5.27
	Kp (cm/h)	5.71E-04	4.3E-05

Table 3
Cumulative penetration

Time	Human (µg/cm²)		Rat (µg/cm²)	
(hours)	Mean	SD	Mean	SD
0.5	4.70	5.53	10.8	2.32
1	5.83	8.29	35.7	6.57
2	10.4	11.9	109.2	29.6
3	15.8	14.0	169.2	45.8
4	22.2	17.5	232.1	85.5
5	27.6	21.2	298.4	75.0
6	35.0	26.8	359.1	76.2
7	43.2	32.8	436.6	84.0
8	46.8	34.7	511.2	97.0
12	71.1	53.4	790.4	118.9
24	164.2	146.5	1628.3	23.3

Figure 1
In vitro static diffusion cell

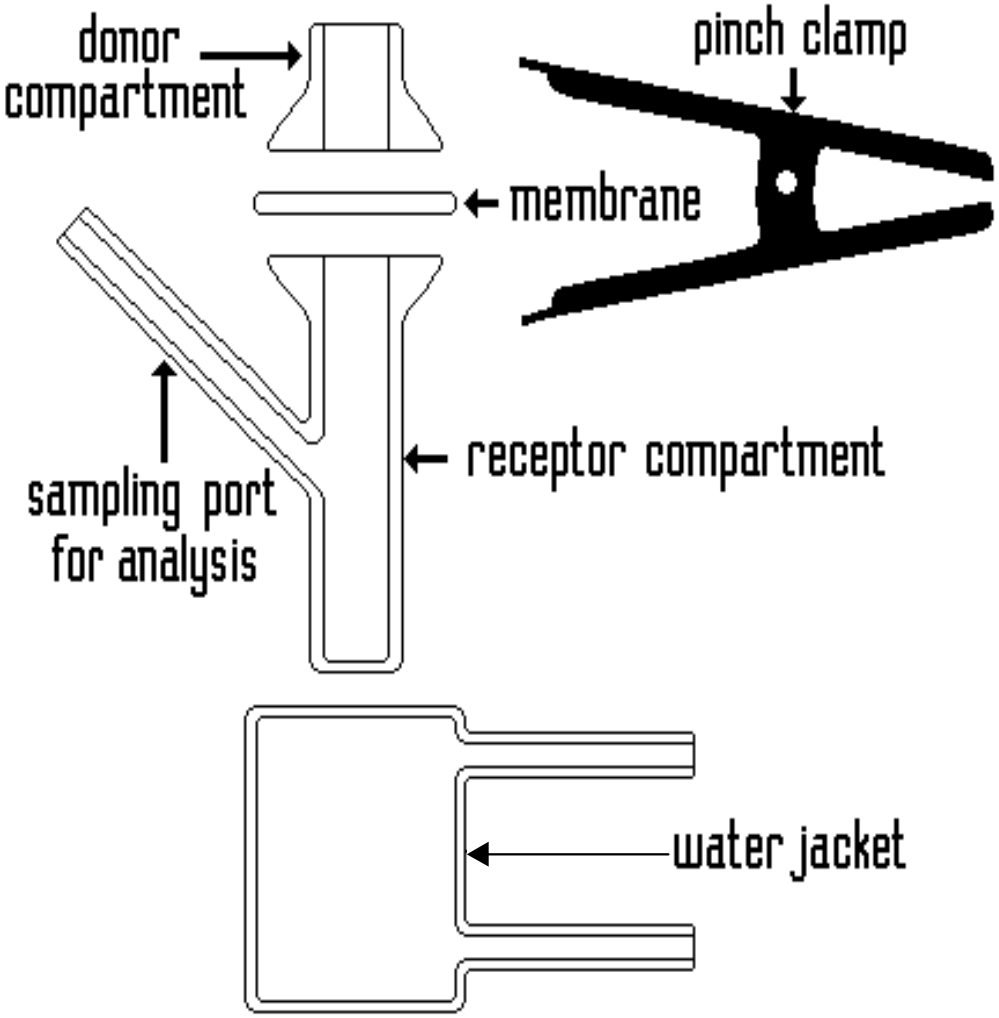

Figure 2
Cumulative penetration